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Evaluation of human neutrophil gelatinase-associated lipocalin level in asthma-COPD overlap syndrome of elderly patients

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ABSTRACT

Aims: Human neutrophil gelatinase-associated lipocalin (NGAL) is a protein considered as a noninvasive prognostic biomarker in Asthma-COPD overlap syndrome (ACO). In this study we aimed to show the usability of NGAL as a biomarker in ACO characterized with airway inflammation in over 65 years old.

Methods: Three patient groups (Group I: COPD, n: 50, Group II: Asthma, n:50, Group III: ACO, n:50) and a healhty non smoker group (Group IV, n:50) are taken into study. The serum NGAL levels of groups are compared. In Group I, II, III the level of serum NGAL is compared with the age, duration of smoking (pocket/year), number of attack per year, respiratory function values, serum C reaktive protein (CRP, mg/dL), serum IgE (IU/mL) and blood neutrophil-to-lymphocyte rate and atopy.

Results: In patients having ACO when the maximum level of serum exist, it is observed that serum NGAL level is increased as the number of attacks is increased ($p<0.002$). On the other hand in the patients having ACO there was also positive correlation between NLR and serum NGAL level ($p<0.05$).

Conclusion: The increase in serum NGAL levels in chronic inflammatory lung diseases suggests that serum NGAL levels can be used as a biomarker to assess inflammation severity

Keywords: Elderly, human neutrophil gelatinase-associated lipocalin, asthma-COPD overlap syndrome

INTRODUCTION

Asthma is a heteregenous chronic airway diseases which leads to reversible airflow obstruction based on innate and adaptive immune responses. Heterogenity of asthma is due to genetic and environmental reasons, chronic aiway diseases affecting 1-18% of the population.[1] In the majority of patients are underlying atopy. Inflammatory cell-derived mediators cause increased vascular permeability, mucosal edema, bronchial smooth muscle hypertrophy and subepithelial fibrosis.[2-6] Chronic obstructive pulmonary disease (COPD); It is a disease characterized by progressive airflow restriction that is not fully reversible. This disease develops as a result of an inflammatory process against harmful gases and particles, especially cigarette smoke. Inflammation is not only limited to the lungs, but also shows systemic features.[7] COPD, which is a preventable and treatable disease, progresses with exacerbations that increase in severity and frequency. Chronic airway obstruction in COPD develops due to narrowing of small airways and parenchymal destruction. Chronic inflammation causes structural changes in the small airways. This inflammatory process and parenchymal destruction lead to loss of alveoli and a decrease in the elastic return pressure of the lungs.[8] Asthma-COPD overlap (ACO) refers to a group of poorly studied and characterised patients reporting with disease presentations of both asthma and COPD, thereby making both diagnosis and treatment challenging for the clinicians.[9-11] ACO is defined in the GINA and GOLD guidelines as "the accompanying of asthma and COPD characteristics in patients with persistent airflow restriction at close rates". ACO can be defined as symptoms accompanying increased airway sensitivity, reversible airway obstruction and persistent airflow restriction. While at present there is no general consensus on definition or defining features for this subgroup of patients, there is broad agreement that they experience frequent exacerbations.[12-14]

The prevalence of ACO is 15-55% in the population ACO has been found to be a disease of advanced age in recent studies. They exhibit a higher burden in terms of both mortality and morbidity in comparison to patients with only asthma or COPD. The pathophysiology of the disease and its existence as a unique disease entity remains unclear.[15,16]

Neutrophil Gelatinase-associated Lipocalin (NGAL), also known as neutrophil glucosaminidase-associated lipocalin, is a 178 amino acid, 25 kDa protein synthesized in neutrophils and epithelial cells. Gene expression has been demonstrated in the

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uterus, prostate, salivary glands, lung, trachea, stomach, colon and kidney. Neutrophil gelatinase-related lipocalin production increases in humans in response to ACO and can be detected in serum at a very early stage.[17-20]

In recent years, in studies conducted in ACO patients, there are limited numbers of publications about the high serum lipocalin levels associated with human neutrophil gelatinosis and that it is associated with the severity of the disease. This study was planned considering that there is a limited amount of literature information in association with NGAL and asthma, COPD and ACO in elderly patients.

METHODS

Ethics

The study was carried out with the permission of University of Health Science Dışkapı Yıldırım Beyazıt Health Pratctice and Research Centers Ethics Committee (Date: 23.03.2015, Decision No: 21.14). The study was planned in accordance with the suggestions of the Helsinki Document and our hospital ethic commission (23.03.2015/21.14). Signed consent forms were obtained from all the patients who volunteered. This study was not supported by any organization. There isn't conflicts of interest, financial or otherwise in this study.

Patients

Two hundred and sixty patients aged 65 years and older (T hey were diagnosed with ACO, COPD and asthma according to the GOLD, GINA 2020 criteria between May 2015 and December 2020, which was done our hospital. However, the full article has not previously been published. Three patient groups (Group I: COPD, n: 70, Group II: Asthma, n: 70), Group III: ACO, n:70) and a healhty non smoker group (Group IV, n:50) are taken into study. Asthma, ACO and COPD was excluded in the control group by demographic data, physical examination and respiratory function tests. Detailed physicals exams, detailed blood tests, height, weight and neck circumference measurements were conducted. The body mass index (BMI, kg/m²) of each patient was calculated by dividing the body weight in kilograms by the square of the patient's height in meters. Assuming its benefit in exclusion of the respiratory system pathologies, posteroanterior chest radiography was performed. Pulmonary function tests were performed in the respiratory laboratory of our hospital, using a Jaeger brand spirometer according to the criteria of American Thoracic Society (ATS). Forced expiratory volume in one second (FEV1, liters), and forced vital capacity (FVC, liters), and FEV1 to FVC (%) values are measured at least 3 times, and optimal values were recorded. Twentyfour of well controlled asthmatic patients were categorized as mild persistant, 46 of them as moderate persistant asthma according to GINA before treatment. The patients also underwent physical examinations. One or more positive results to allergens in skin prick test (Allergopharma, Germany) was accepted as atopy. According to previous skin prick test results of patients regullary followed in our clinics, 15 asthma patients (3 mild peristant, 9 moderate persistant) and three ACO patients had atopy.

The severity of the COPD was categorized into moderate (n: 31, FEV1/FVC<0.70,50%≤FEV1<80% predicted), and severe (n:39, FEV1/FVC<0.70, 30%≤FEV1<50% predicted) according to GOLD criteria. Patient with ACO was categorized according to GINA and GOLD criteria.[20] The treatment of our patient's protocols was explained to GOLD and GINA criteria. Anemia was defined as Hb<12 g/dl in male. Baseline arterial oxygen saturation was defined ≤88%. Our exclusion criteria were an acute COPD, ACO and exacerbations in the last 3 months (increased cough, dyspnea, sputum production, and/or purulence), other acute and chronic diseases, a blood transfusion in the last 6 months, anti-inflammatory therapy (oral, parenteral systemic glucocorticosteroids) within the last 3 months, and anemia within the last 6 months, malignancy and pregnancy.

Laboratory

In all patient groups and control group included in the study, blood and serum samples for full blood testing were taken in the morning on an empty stomach. Serum CRP (0-6 mg/L), serum Ig E (0-180 IU/mL), serum neutrophil (%), lymphocyte (%) levels were assessed using standard laboratory methods. The blood sample drawn for the measurement of NGAL were collected early in the morning after a minimum of 10 hours fasting and centrifuged for 10 min at 3000 rpm, and serum was stored at -80°C in aliquots until the day of analysis. Serum NGAL levels were measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), according to the instructions of the producer company (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China). Results were given as ng/mL. The intra-assay coefficient of variation (CV) was <10% and the inter-assay CV was 12%. Chest radiographs were evaluated to exclude other pathologies. The NLR was obtained by dividing the absolute number of neutrophils by the absolute number of lymphocytes. The data were expressed as a percentage value (i.e.: 2.25%).

Statistical Analysis

Statistical analyses were performed using (Predictive Analytics SoftWare) version 20. Descriptive results for numerical variables were expressed as mean ± standard deviation; categorical variables were expressed as frequency and percentage. Chi-square analysis or Fisher's exact test, where appropriate, was used to compare proportions in different groups. There was said to be a statistically significant difference between groups when P<0.005. In that case, difference groups were determined by the Mann-Whitney U test and Bonferroni correction.The relationship of serum NGAL levels with other variables was examined using the Spearman Correlation coefficient.

RESULTS

Demographic and laboratory data for the 260 patients and control group monitored at our clinic were demonstrated in **Table 1**. The mean serum NGAL level of the control group was found to be 211.210 (102.85-800.49) ng/ml. Serum NGAL level of asthmatic patients was 176.280 (82.78-976.59) ng/ml while the mean serum NGAL level of the patients with COPD was found to be 221.901 (97.84-1938.62) ng/ml and with ACO was found to be 269.146 (153.70-1796.78). There was a significant difference between serum NGAL levels of ACO patients (p=0.002). Except ACO group, all of the patient groups and control group included in the study had C-reactive protein (CRP) levels within the normal limits (upper normal level, 6 mg/L). **Figure 1** shows the box plot graphs of serum NGAL level in all patients groups. **Figure 2** shows tho box plot FEV1(L) level of patients and control group. **Figure 3** shows the box plot FEV1/FVC (L) level of patients and control group. Forced expiratory volume in one second (FEV1) and FEV1/FVC ratio was higher patients with asthma and control groups. **Figure 4**

shows the box plot acute exacerbation of patients with Asthma, COPD, ACO. Acute exacerbation was also high in patients with ACO. Relationships between the serum NGAL levels and other variables were demonstrated in **Table 2**. The serum NGAL levels was found significantly higher in patients with ACO patients compared to other groups. It was found that this height was positively correlated with number of attack per year ($p<0.05$). On the other hand in the patients having ACO there was also positive correlation between NLR and serum CRP level and serum NGAL ($p<0.05$). There was no other relation found among the other variables. **Figure 5** shows the box plot graphs of the relationship between smoking and serum NGAL level in patients with ACO.Serum NGAL was increasing in proportion to smoking.But not statistically significant ($p>0.05$).

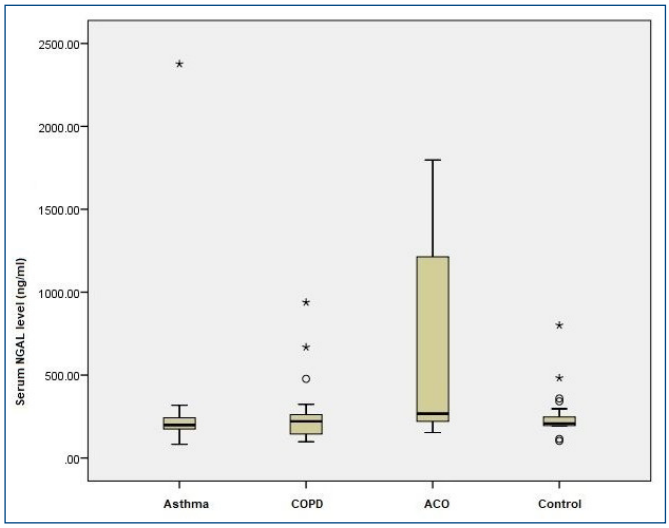

Figure 1. The box plot graphs of serum NGAL level in all patients groups

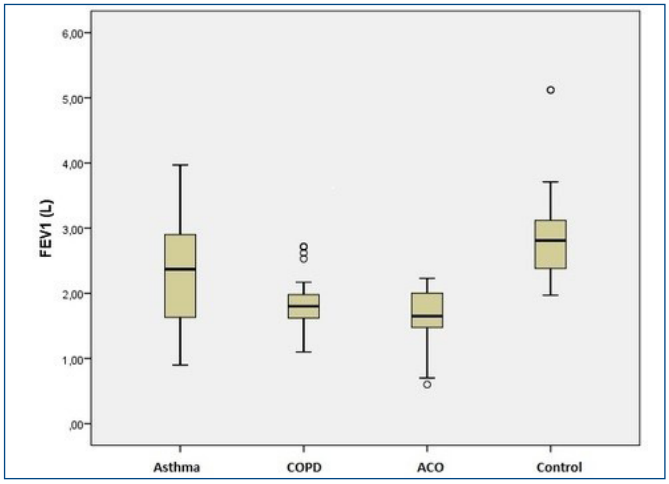

Figure 2. FEV1(L) level of patients and control group.

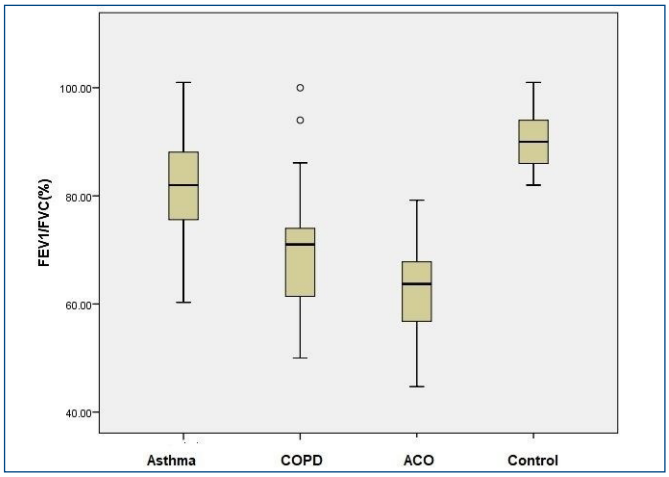

Figure 3. The box plot FEV1/FVC (L) level of patients and control group.

Table 1. Demographic information and laboratory characteristics of patient groups and control group.

Variables	Asthma (n:70)	COPD (n:70)	ACO (n:70)	Control (n:50)
Age (year)	65.10 ± 10.98	69.80 ± 11.30	74.06 ± 11.57	65.06 ± 15.57
Sex (Female/Male)	47/23	28/42	31/39	33/17
Smoking (pack-year)	-	32.91 ± 23.11	25.50 ± 8.51	-
Dieasase year	12.40 ± 3.39	13.76 ± 5.06	11.03 ± 5.62	-
Exacerbation (/year)	1 (0–2)	2 (1–4)	3 (2–4)	-
BMI (kg/m²)	27.34 ± 3.70	26.49 ± 4.64	26.19 ± 4.09	28.29 ± 5.09
FEV1/FVC (%)	72.45 ± 7.65	60.34 ± 12.08	48.17 ± 11.67	92.45 ± 7.75
FEV1(%)	73.36 ± 6.48	65.20 ± 6.30	50.12 ± 6.13	90.36 ± 5.47
Total IgE level (1–83 IU/ml)	137.60 (47-1211)	48.9 (10–81)	151 (125–1825)	21 (9–67)
Atopy (+)	15			
C-reactive protein (CRP, 0–6 mg/L)	5.4 ± 3.5	7.9.±4.1	10.7 ± 5.7	3.9 ± 2.8
Neutrophil (×10³/mm³)	4.05 ± 2.53	4.91 ± 1.53	6.25 ± 3.03	4.70 ± 1.71
Lymphocyte (×10³/mm³)	1.08 ± 0.66	1.45 ± 0.73	1.71 ± 0.42	1.91 ± 0.24
Blood neutrophil to lymphocyte ratio	2.92 ± 1.69	3.22 ± 1.06	4.37 ± 6.09	2.72 ± 1.44
Serum HNGAL level (ng/ml)	176.280 (82.78-976.59)	221.901 (97.84-1938.62)	269.146 (153.701–796.78)	207.110 (102.85-800.49)

Table 2. Relationships between the serum NGAL levels and other variables.

Variables	Asthma (n:70)		COPD (n:70)		ACO (n:70)	
	r	p	r	p	r	p
Age (year)	0,315	0,085	0,191	0,369	0,061	0,731
Disease year	0,102	0,591	0,337	0,070	0,158	0,404
Exacerbation (/year)	-,189	,327	,334	,077	0,248**	0,021
BMI (kg/m²)	0,159	0,402	0,067	0,724	0,160	0,400
FEV1/FVC (%)	0,025	0,899	0,193	0,311	0,235	0,237
FEV1(%)	0,031	0,831	0,147	0,387	0,185	0,358
Total IgE level (1–83 IU/ml)	0,168	0,398	0,193	0,307	0,234	0,129
Blood neutrophil to lymphocyte ratio	0,162	0,407	0,033	0,841	0.280**	0,012
C-reactive protein (CRP, 0–6 mg/L)	-0.01	0.9	-0.04	0.7	0.614**	0.011
Atopy (+)	-0.02	0.7	-	-	0.22	0.091

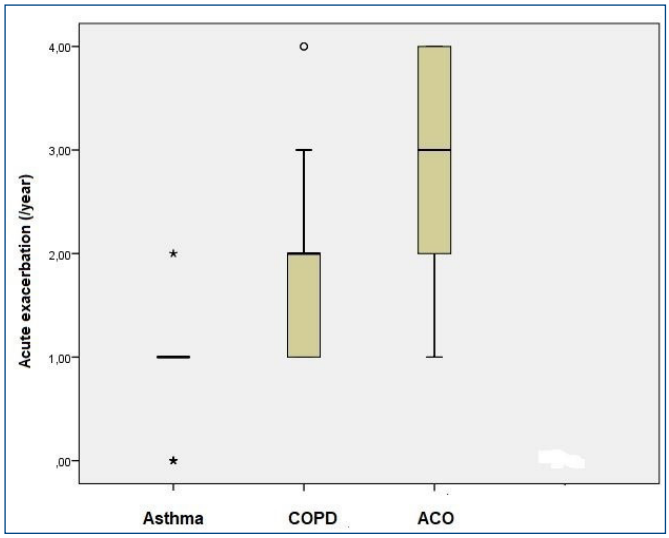

Figure 4. The box plot acute exacerbation of patients with Asthma, COPD, ACO.

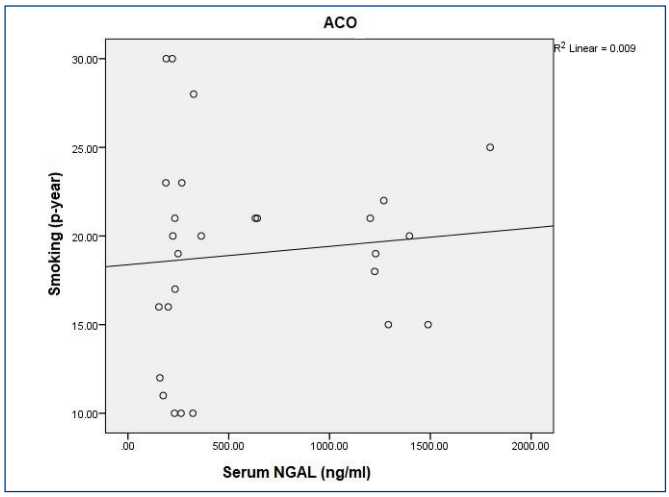

Figure 5. The box plot graphs of the relationship between smoking and serum NGAL level in patients with ACO.

DISCUSSION

The aim of this study is to compare and analyse serum NGAL levels at chronic inflamatuar lung disease such as asthma, COPD, ACO and to evaluate the clinical utility of serum NGAL as a predictive marker for these disease for elderly patients. In this study, we found that serum NGAL level was higher in ACO group than COPD, asthma and control groups. Moreover, serum NGAL level is increased as the number of attacks is increased (p: 0.021) with ACO patients. On the other hand in the patients having ACO, there was also positive correlation between blood NLR and serum CRP levels with serum NGAL levels. There was no correlation found among the other variables. Chronic inflammatory lung diseases such as COPD, asthma and ACO are among the commonly seen reasons of death worldwide. Studies have shown that during stable period and exacerbations in patients with chronic inflammatory lung diseases increase both local and systemic inflammatory response. In recent years, serum NGAL in inflammation, endothelial dysfunction, angiogenesis and remodeling have been reported to increases. Until now, NGAL's research continues as an inflammatory marker in the various diesases. NGAL is expressed at low levels in different tissues, such as kidney, trachea, lungs, stomach, and colon, and its expression increases noticeably in inflammation. Chronic inflammatory lung diseases are one of them.[21-23]

There has been limited number of studies related with NGAL in lung diseases. In first studies, serum NGAL is significantly elevated in the bronchoalveolar lavage fluid (BALF) and induced sputum of COPD patients. In normal human lung tissue, NGAL is constitutively expressed within tracheal goblet cells and in type II pneumocytes. An increase in the level of inflammation occurs with the progression of chronic inflammatory lung diseases. In our study, the detection of increased serum NGAL levels in elderly patients with chronic inflammatory lung diseases in the stable period supported that, rather than being restricted to the lung therefore being local, they are systemic inflammatory diseases.[24-26] In previous studies, it was reported that serum NGAL expression increased in various malignancies and it is a prognostic factor in these cancers and can be used in follow-up, after treatment. After acute ischemic and nephrotoxic injury, serum NGAL expression from the renal epithelium increases in response to inflammation. Serum NGAL exists as an acute phase protein based on high levels in serum, epithelial, urine and fecal levels of patients with active inflammatory disease.[27-30] There is no sufficient data on serum NGAL levels during exacerbation periods in ACO. Our study showed serum NGAL in elderly patients having ACO when the maximum level of serum exist, it is observed that serum NGAL level is increased as the number of attacks is increase. Our findings are supported by the other studies for higher serum levels of NGAL in elderly patients. We found serum NGAL level to be highest in ACO as compared to asthma, COPD and controls. Our results are in good agreement with reports suggesting higher levels of NGAL in ACO as compared to asthma. Further, a significant negative correlation was observed between serum NGAL and lung function in ACO patients. Our findings are supported by the work of Gao, et al, where an increased NGAL expression in sputum has been independently correlated with degree of airflow limitation in ACO.[24]

Studies have shown mice lacking serum NGAL gene are more sensitive to some gram (-) bacteria. In addition, mortality rates were found to be higher in sepsis compared to normal mice. Therefore, serum NGAL is defined as an essential component of innate immunity against bacterial infections. NGAL production increases in bacterial infections and NGAL levels in tissues are used to differentiate bacterial-viral infections. Activated neutrophils secretes matrix metalloprotease 9 (MMP-9). NGAL binds to MMP-9 and prevents prolonged collagen degradation. These actions of NGAL are of particular interest in relation to patients with chronic inflammatory lung diseases especially development of emphysema and airway wall remodeling.[31-33] In a study on NGAL, sputum levels of NGAL were significantly higher in patients with ACO compared with subjects without ACO and that level of NGAL was related to important ACO characteristics.[34] NGAL is actively secreted from both neutrophils and epithelial cells. The upregulation of NGAL transcription in neutrophils and epithelial cells involves fundamental upstream inflammatory pathways, such as activation of nuclear factor- k B, interleukin-1 activation, and interaction between microbial products and certain toll-like receptors. NGAL may be a marker of several upstream inflammatory pathways, reflecting several processes potentially involved in the pathogenesis of COPD and ACO . In our study, we

found that both NLR and exacerbation rate were positively correlated with increasing serum NGAL level, which supports the relationship between increased neutrophil rate and NGAL in inflammation especially patients in ACO.

Systemic inflammation in ACO is similar to COPD. Serum NGAL value was found close to each other in ACO and COPD groups in our study. This result may support the similarity of inflammatory events between COPD and ACO. In another study, the sputum levels of NGAL were significantly increased in ACO when compared with COPD and asthma groups. The sputum NGAL levels might be related to airway inflammation and low-grade microbial colonization, which predispose patients with ACO to acute viral infections and exacerbations.

The present study aimed to investigate the potential of NLR, as a prognostic markers in patients with chronic inflammatory lung diseses. It has been demonstrated that NLR may be a useful predictor of systemic inflammation and with prognosis of many cardiovascular diseases, malignancies and chronic inflammatory diseases.[35-37] Taking this idea into account, some studies focused on NLR which are considered among the markers of systemic inflammatory response for COPD, ACO and asthma. One of these have shown that increase in the ratio of circulating blood NLR levels may serve as a marker of systemic inflammation for ACO, COPD patients. The patients with stable disease and healthy controls have shown lower NLR values than the patients with COPD exacerbation, according to the study done by Lee, et al.[38] In another study by Lee et al, they found NLR values increased with restriction of airflow limitation. They tought that the change in NLR was due to COPD itselfs.[39] So far, the studies reviewed showed that can be used to predict COPD prognosis and mortality. In another study, neutrophils and NLR, as indicators of circulating immune complexes, were found higher in patients with COPD and ACO than those in the healthy population. They thougt that NLR may be used as a biomarker to distinguish and diagnose COPD and ACO.[40] With a different perspetcive, Li et al.[41] they found the level of NLR similar in COPD and ACO patients. In another study found that the NLR ratio increased in parallel with the severity of COPD.[42] Similar results were found in our study. The NLR ratio increased with the aggravation of inflammation. Our patient with ACO groups had high serum CRP levels. Ultimately ACO and COPD are associated with systemic inflammation. The increase NLR and CRP may supports this situation.[43-47] Due to the high number of exacerbation period for ACO, we think that the role of inflammatory mechanisms may increase in this desase.

CONCLUSION

As a result; especially in elderly patients, there is no known prognostic biomarker for ACO. In patients with chronic inflammatory lung diseases, NGAL may be a systemic useful biomarker reflecting the inflammation level. In additional to chronic systemic inflammation may explain an parellel increase in NLR value, CRP and serum NGAL. This study has some limitations. Additional studies are needed for clinical evaluation of the progression of chronic inflammatory lung diseases. As our study was conducted with a small sample group, the study should be supported with larger scale studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Science Dışkapı Yıldırım Beyazıt Health Practice and Research Centers Ethics Committee (Date: 23.03.2015, Decision No: 21.14).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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